ORIGINAL RESEARCH

Microfiber ingestion in *Patagonotothen kreffti* from the Namuncurá/ Burdwood Bank Marine Protected Area

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ABSTRACT. Microfibers (MFs) are an anthropogenic pollutant with a major impact on the marine environment. Due to their size, they can be ingested directly (from the environment) or indirectly (with their prey) by organisms. The aim of this study was to analyze and compare the occurrence, abundance and physicochemical characteristics of MFs present in *Patagonotothen kreffti* from the Marine Protected Area Namuncurá/Burdwood Bank (MPA N/BB) during two seasons: winter and spring. The notothenioids were obtained from the oceanographic surveys of August 2018 and December 2018. Oxidative digestions of fish gastrointestinal tracts were used to recover MFs and further determine the number per individual (MFs $ind.^{-1}$) and per gram of tissue (MFs g^{-1}). Each MF was characterized by total length, color, wear and discoloration, and chemical composition. In winter, *P. kreffti* presented an occurrence of 100% with values of 5.71 ± 3.19 MFs $ind.^{-1}$ and 1.22 ± 1.45 MFs g^{-1}, while in spring the occurrence was 85.71%, with abundances of 3.71 ± 3.30 MFs $ind.^{-1}$ and 0.28 ± 0.23 MFs g^{-1}, with no significant differences between seasons (Wilcoxon, $p > 0.05$). The mean size of the MFs was 0.89 mm, and blue MFs were in the majority (57.35%). Wear and discoloration were observed in 42.42% of the MFs. Cellulose MFs were the most abundant followed by polyester MFs. *P. kreffti* could be used to assess MF contamination in the N/BB MPA because it is endemic and has higher abundances of MFs than other notothenioids in the area.

Key words: Notothenioids, semi-synthetic microfibers, synthetic microfibers, cellulose, bioindicator.

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Ingestión de microfibras en *Patagonotothen kreffti* del Área Marina Protegida Namuncurá/ Banco Burdwood

RESUMEN. Las microfibras (MFs) son un contaminante antropogénico con gran impacto en el ambiente marino. Debido a su tamaño, pueden ser ingeridos por los organismos directamente (desde el medio ambiente) o indirectamente (con sus presas). El objetivo de este estudio fue analizar y comparar la ocurrencia, abundancia y características fisicoquímicas de MFs presentes en *Patagonotothen kreffti* del Área Marina Protegida Namuncurá/Banco Burdwood (AMP N/BB) de dos temporadas: invierno y primavera. Los nototénidos fueron obtenidos de las campañas oceanográficas de agosto y diciembre 2018. Mediante digestiones oxidativas de los tractos gastrointestinales, se recuperaron las MFs y determinó el número por individuo (MFs $ind.^{-1}$) y por gramo de tejido (MFs g^{-1}). Se caracterizó a cada MF con longitud total, color, desgaste y decoloración, y la composición química. *P. kreffti* en invierno presentó una ocurrencia del 100% y valores de 5,71 ± 3,19 MFs ind.$^{-1}$ y 1,22 ± 1,45 MFs g^{-1}, mientras que en primavera la ocurrencia fue 85,71%, y las abundancias de 3,71 ± 3,30 MFs ind.$^{-1}$ y 0,28 ± 0,23 MFs g^{-1}, sin hallarse diferencias significativas entre temporadas (Wilcoxon, $p > 0,05$). El tamaño promedio de las MFs fue 0,89 mm, las MFs azules fueron mayoritarias (57,35%). Se observó desgaste y decoloración en 42,42% de las MFs. Las MFs de celulosa fueron las más abundantes seguidas por las de poliéster. *P. kreffti* podría facilitar la evaluación de las MFs en el AMP N/BB debido a que es endémica y presenta abundancias de MFs mayores a otros nototénidos del área.

Palabras clave: Nototénidos, microfibras semi-sintéticas, microfibras sintéticas, celulosa, bioindicador.

INTRODUCTION

The scientific community around the world is becoming increasingly concerned about anthropogenic pollution of the marine environment. Anthropogenic particles (APs) are particles of manufactured origin present in the environment, including synthetic particles such as plastics, and semisynthetic particles such as those derived from the textile industry or tire wear (Barrows et al. 2018; Athey et al. 2020; Gaylarde et al. 2021). Smaller-sized APs (< 5 mm) are emerging contaminants that pose an increasing threat to both wildlife and human health. Furthermore, their ubiquity and persistence in the environment have raised concerns about their impact on marine biodiversity (Adams et al. 2021).

Microfibers (MFs; 0.001-5 mm) are the most abundant APs and are currently one of the main marine pollution issues (Gago et al. 2018; Suaria et al. 2020; Di Mauro et al. 2022). Due to their small size, these thread-like particles composed of synthetic (e.g. polyester) or semisynthetic materials (e.g. manufactured/modified cellulose) can be ingested by organisms of different trophic levels, either directly from the environment or indirectly through their prey (Gago et al. 2018). Ingestion of MFs has been reported in various species of marine invertebrates and vertebrates, including several fish species across different regions (e.g. Zhang et al. 2021; Sanchez-Guerrero-Hernandez et al. 2023; Ojeda et al. 2024). Experimental studies on aquatic fauna have reported a range of negative effects, including reduced growth rates, neurotoxicity, metabolic disturbances, cytotoxicity, and endocrine, immune, and reproductive dysfunctions (de Sá et al. 2018; Barboza et al. 2020; Medriano and Bae 2022; Barboza et al. 2023). Understanding these impacts on fish is especially important, as they play a key role in ecosystems by contributing to energy and nutrient dynamics, as well as modifying habitats (Villéger et al. 2017, Marina et al. 2022). Given their ecological relevance and accessibility for sampling, fish are considered proper bioindicators of APs ingestion, and several species have been used to assess APs intake in marine environments (Alves et al. 2024; Santonicola et al. 2024).

The Burdwood Bank (BB) is a shallow seamount in the southwestern Atlantic Ocean, located 150 km east of Isla de los Estados, 200 km south of the Malvinas Islands and 1,200 km from South Georgia Island (Schejter et al. 2016; Falabella et al. 2017). This region is influenced by sub-Antarctic waters and upwelling areas, and exhibits high abundance and richness of organisms (e.g. Schejter et al. 2016; Delpiani et al. 2020; Schejter and Albano 2021). Due to its ecological and oceanographic importance, this region was declared as the first oceanic Marine Protected Area in Argentina, ‘Namuncurá I’ (established in 2013), with the addition of the region’s deep slope, ‘Namuncurá II’, in 2018.

The genus *Patagonotothen*, which is primarily represented by *Patagonotothen guntheri* (Norman, 1937), *P. ramsayi* (Regan, 1913), *P. elegans* (Günther, 1880), and *P. cornucola* (Richardson, 1844), has the highest specific richness among the ichthyofauna of the Marine Protected Area Namuncurá/Banco Burdwood (MPA N/BB) (Delpiani et al. 2020). Of particular interest is *P. kreffti* Balushkin and Stehmann 1993, which is regarded as a sister species to *P. ramsayi*. The geographical distribution of *P. kreffti* is limited to the BB region (Álvarez Oyarzo 2020).

Previous studies in the MPA N/BB reported higher MFs values in the water column compared to regions known as accumulation hotspots, such as the Gulf of Mexico and the Arctic Ocean (Di Mauro et al. 2022). The presence of MFs and other APs was also reported in asteroids (Cossi et al. 2021), as well as in notothenioids *P. guntheri* and *P. ramsayi* (Ojeda et al. 2024), two key species in the communities of the MPA N/BB (Delpiani et al. 2020). Given the unique fish species restricted to the BB area, the objective of this study was to analyze the occurrence, abundance, and chemical nature of MFs found in the gastrointestinal tract of *P. kreffti* in two seasons (winter and spring) at the MPA N/BB.

MATERIALS AND METHODS

Study area and sampling

Samples were obtained in the MPA N/BB (54° S-55° S, 56° W-62° W) from oceanographic surveys conducted in August (austral winter) and November (austral spring) of 2018 on the vessels ARA 'Puerto Deseado' and 'BIP Victor Angelescu', respectively (Figure 1). A total of 50 fishes were collected using a demersal bottom trawl pilot net in August (S1: 54° 32′ 26″ S, 59° 24′ 46″ W, 133 m, N = 20) and November (S2: 54° 19′ 20″ S, 60° 24′ 14″ W, 118 m, N = 30) for APs determination. Fish were measured and dissected. The gastrointestinal tract of each specimen was meticulously removed and preserved individually in aluminum foil envelopes at -20 °C until processing. Specimens were also preserved at -20 °C and subsequently identified by molecular genetic methods (Álvarez Oyarzo 2020). Following genetic identification, 14 specimens of *P. kreffti* were selected to study (August N = 7, total length range: 210-302 mm; and November N = 7, total length range: 220-282 mm).

Quality assurance/quality control

All materials and equipment used for the extraction of MFs were thoroughly cleaned before processing each sample, including washing and rinsing with Milli-Q water, and then allowed to dry beneath aluminum foil. Laboratory materials were inspected under a stereomicroscope to confirm the absence of residual contamination. Glassware, white cotton laboratory coats, sterile Petri dishes and nitrile gloves were utilized. Prior to processing each sample, laboratory benches were meticulously cleansed with 96% ethanol. Furthermore, to minimize the potential for cross-contamination, laboratory personnel movement was restricted. Every time a set of seven samples was processed simultaneously, a procedural blank (control without a fish gastrointestinal tract) was included. Following filtration, filter papers were promptly transferred to a covered Petri dish. At all processing stages, a clean Petri dish was placed adjacent to the samples and then examined in order to monitor for airborne contamination.

Microfibers isolation

The extraction method employed for MFs was a modification of Pérez et al. (2020). Aluminum foil was removed from each individually preserved sample, after which digestive tracts were placed in Petri dishes and weighed immediately while still frozen using an electronic precision balance (± 0.001 g). Digestive tracts of each specimen were then placed in glass bottles with H_2O_2 (30% v v^{-1}, 1:10 w v^{-1}) and incubated at 55 °C for 72 h in darkness. Subsequently, bottles were shaken mechanically for 1 h at room temperature. Finally, each digested sample was incubated at 60°C for 30 min followed by vacuum filtering through a filter paper of 22 μm pore size (Whatman no 541).

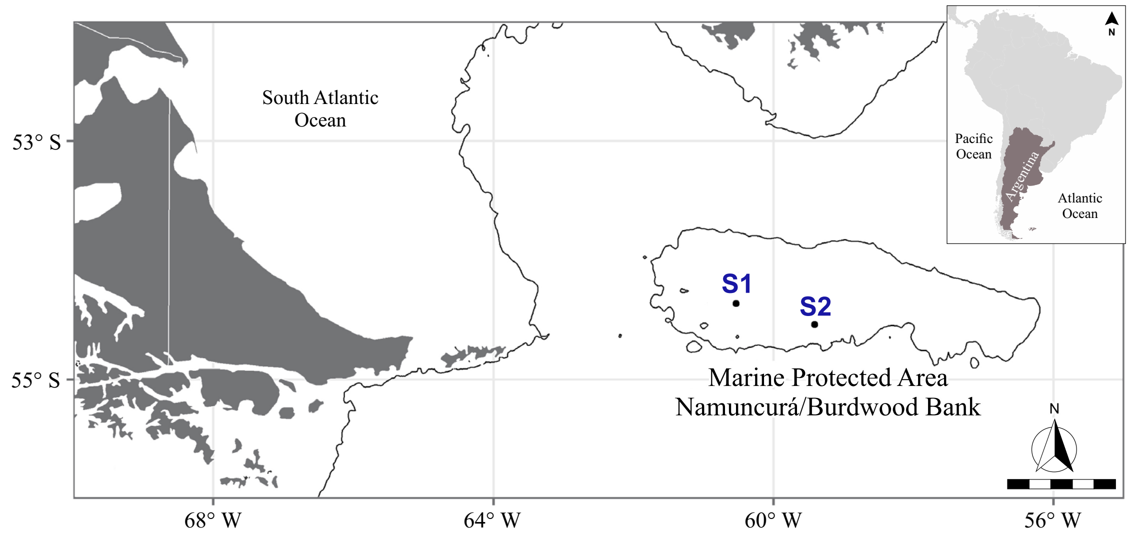

Figure 1. Sampling sites in the Marine Protected Area Namuncura/Burdwood Bank during 2018. Circles represent sampling stations. S1: August, 118 m (54° 22′ 34″ S, 60° 54′ 15″ W); S2: November, 133 m (54° 32′ 26″ S, 59° 24′ 46″ W).

Identification and quantification of microfibers

Filters were observed under a stereomicroscope NIKON SMZ 800, always in covered glass Petri dishes to prevent air contamination. The MFs were imaged using a digital camera attached to the stereomicroscope. The MFs were subsequently enumerated and classified according to their color and the presence of deterioration, including wear and discoloration (Hidalgo-Ruiz et al. 2012; Vinci et al. 2021). The number of MFs in the samples was adjusted when similar MFs were found in control or procedural blanks (two microfibers were discarded from one individual; supplementary material, Table S1). The maximum length of each MF was measured using ImageJ software. For each sample, the total number of MFs per individual (MFs ind.$^{-1}$) and per gram of digestive tract (MFs g^{-1}) was quantified.

A total of 72.72% of the observed MFs were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. The remaining particles could not be analyzed due to methodological constraints, such as instrumental and inherent procedural limitations. The chemical composition of MFs was assessed using a Nicolet iN10 infrared microscope, provided with a highly sensitive liquid-nitrogen-cooled mercury cadmium telluride detector. All spectra were recorded in transmittance mode with a resolution of 8 cm^{-1} in the range of 675 to 4,000 cm^{-1} and compared against HR Nicolet and Hummel Polymer libraries available in the Omnic 9.11.721 software, and a personal library created from spectra of pure polymers obtained under the same conditions.

Data analysis

Spring-winter variation in MFs per individual (MFs ind.$^{-1}$) and per gram of digestive tract (MFs g^{-1}) was analyzed using the non-parametric Mann-Whitney Wilcoxon (WMW). This analysis was conducted subsequent to the examination of normality (Shapiro-Wilk test) and homoscedasticity of variance (Levene's test) assumptions, and there was no evidence of rejection of the null hypotheses. Lengths of MFs for each season were analyzed using a class frequency Fisher's exact test. Statistical significance levels were set at 0.05. The statistical analysis of the data was conducted using the RStudio version 4.2.1 software.

RESULTS

A total of 66 MFs were recovered from the gastrointestinal tract of fish. *Patagonotothen kreffti* from winter showed an occurrence of 100% (40 total MFs) and mean values of 5.71 ± 3.19 MFs ind.$^{-1}$ and 1.22 ± 1.45 MFs g^{-1}, while *P. kreffti* from spring presented an occurrence of 85.71% (26 total MFs) and mean abundances of 3.71 ± 3.30 MFs ind.$^{-1}$ and 0.28 ± 0.23 MFs g^{-1}. There were no significant differences found between seasons (MFs ind.$^{-1}$: WMW, W = 13, p = 0.151; MFs g^{-1}: WMW, W = 37, p = 0.128) (Figure 2 A and B).

The mean length of MFs was 1.00 ± 0.95 mm in winter fish and 0.88 ± 0.77 mm in spring fish. The majority of MFs were found to be less than 1.25 mm during the winter and spring seasons, with percentages of 79.49% and 76.00%, respectively. The class frequency of MFs did not show significant differences between seasons (Fisher's Exact Test, p = 0.116) (Figure 3; supplementary material, Table S1).

The most prevalent color of MFs was blue (winter: 65.00%, spring: 50.00%; Figure 4 A and B; supplementary material, Table S1), followed by black (winter: 27.50%, spring: 26.90%, Figure 4 A and C; supplementary material, Table S1). Red MFs were found in lower proportions (winter: 7.50%, spring: 11.50%, Figure 4 A and D; supplementary material, Table S1) and transparent MFs (11.50%, Figure 4 A and E; supplementary material, Table S1) were only observed in spring fish. The characteristic of wear (Figure 4 B) was observed in 17.50% and 38.46% of the MFs in winter and spring, respectively, and discoloration (Figure 4 D) was found in 15.00% (winter) and 19.23% (spring) of the MFs.

The MFs analyzed by infrared microscopy found in *P. kreffti* from both seasons were semi-synthetic (71.00% of MFs) and synthetic (29.00% of

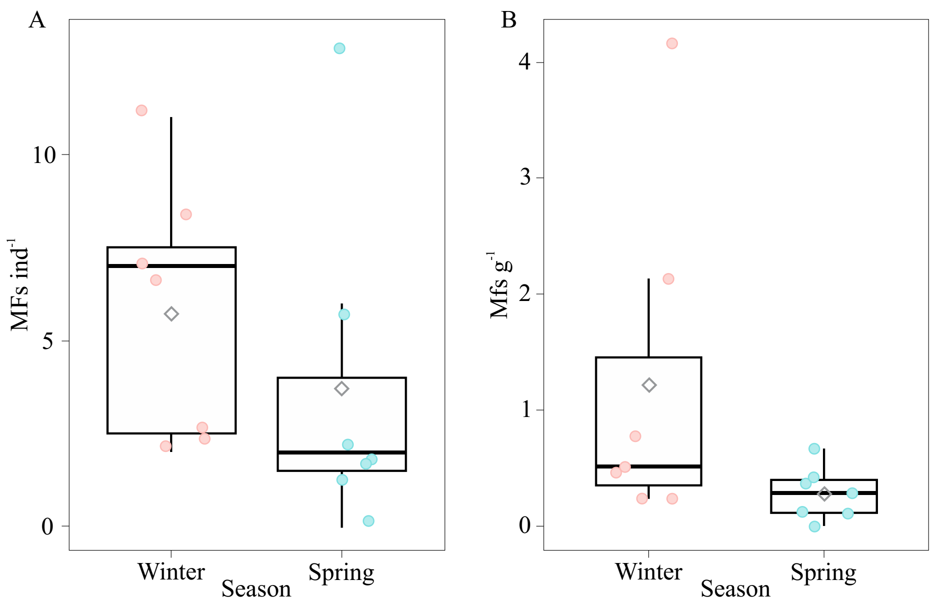

Figure 2. Abundance of microfibers found in digestive tracts of *Patagonotothen kreffti* during two seasons (winter and spring) of 2018 in the Marine Protected Area Namuncurá/Burdwood Bank. A) Total number of microfibers per individual (MFs ind.$^{-1}$). B) Total number of microfibers per gram of digestive tract (MFs g^{-1}). Box plots show the mean (white diamond), median (solid line), first and third quartiles (boxes), minimum and maximum values (whiskers) and values of MFs found in individuals in pink dots for winter and light blue for spring.

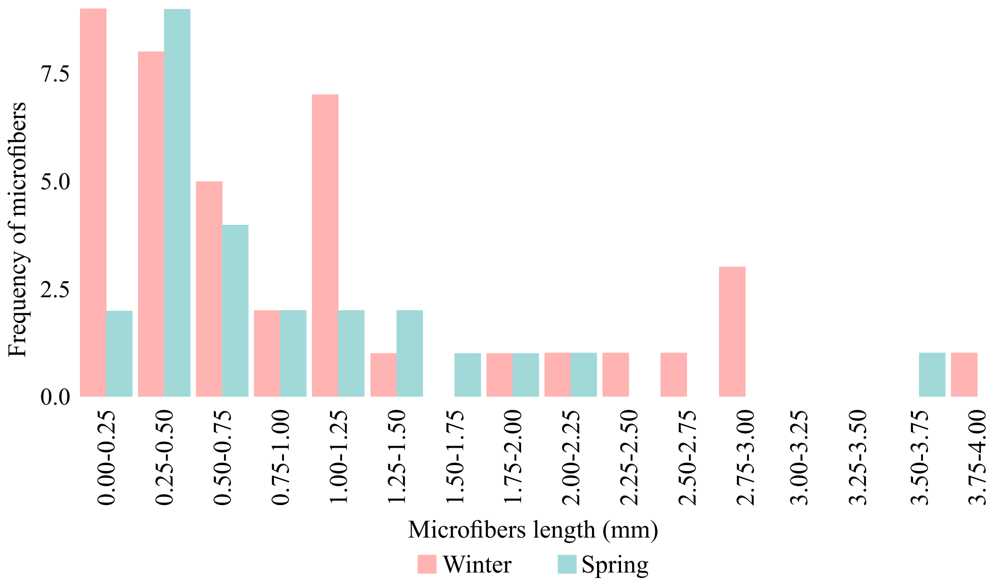

Figure 3. Size distribution of microfibers found in digestive tracts of *Patagonotothen kreffti* during two seasons (winter and spring) of 2018 in the Marine Protected Area Namuncurá/Burdwood Bank.

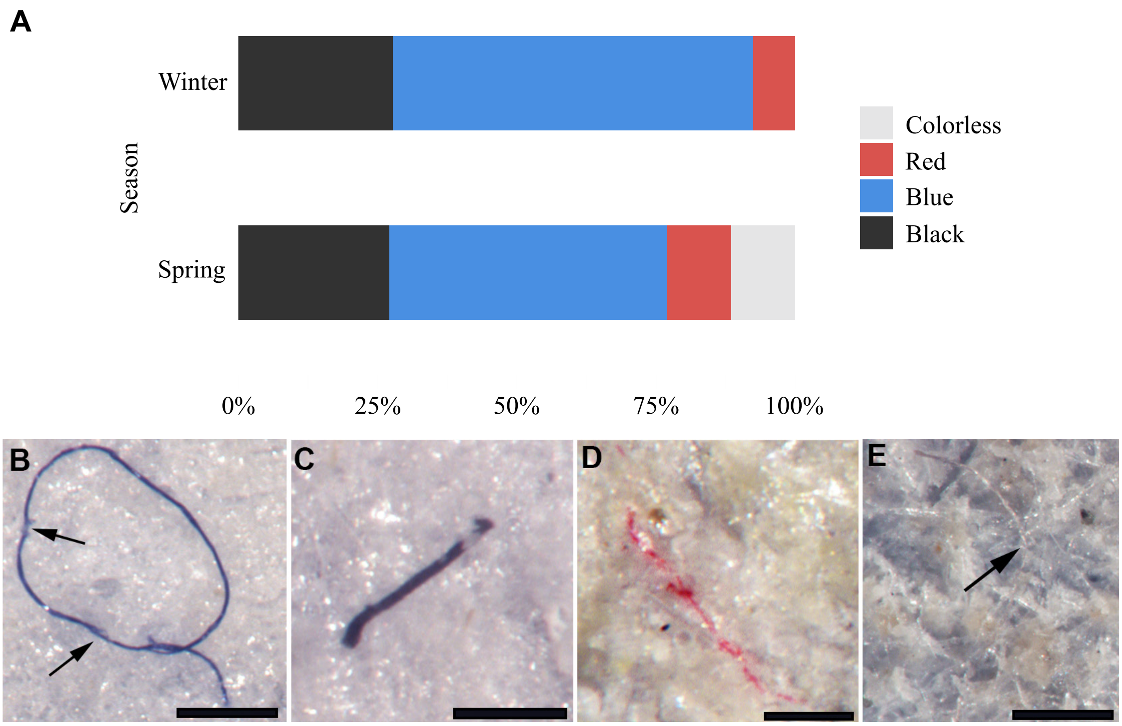

Figure 4. A) Proportion (%) of microfibers colors found in digestive tracts of *Patagonotothen kreffti* during two seasons (winter and spring) of 2018 in the Marine Protected Area Namuncurá/Burdwood Bank. B) Blue microfiber, arrow indicates signs of wear. C) Black microfiber. D) Red microfiber with signs of discoloration. E) Transparent microfiber (arrow). Escale: 150 µm.

MFs). Semi-synthetic MFs were predominantly cellulose (winter: 75.00%, spring: 54.00%) and cellulose-polyamide blend (winter: 8.33%, spring: 4.17%). Synthetic MFs of polyester were found in fish from both seasons (8.33% in winter and 37.50% in spring). Other synthetic MFs, such as polyacrylonitrile (PAN; 4.17%) and polypropylene (4.17%), were identified in winter fish, while polyethylene (4.17%) was found in spring fish (Figure 5; supplementary material, Table S1).

DISCUSSION

The notothenioid *P. kreffti* at MPA N/BB has a high frequency of occurrence of MFs, which is consistent with findings reported for MFs in other marine fishes from the South Atlantic and other regions. *Patagonotothen ramasayi* and *P. guntheri* from MPA N/BB had high occurrences of APs, with MFs being the majority (71.2% and 87%, respectively; Ojeda et al. 2024). Concurrently, in the estuary of Bahía Blanca and Río de la Plata (Argentina), *Micropogonias furnieri* (Desmarest, 1823) exhibits 80% and 100% occurrences of APs, respectively, with predominance in MFs (Arias et al. 2019; Mandiola et al. 2022). Globally, although with variations, numerous studies report high frequencies (68-100%) of MFs occurrences in fish digestive tracts (Pozo et al. 2017; Clere et al. 2022; Santonicola et al. 2024; Santonicola et al. 2025).

Although numerous studies have reported the presence of MFs in fish digestive tracts across different marine regions, the lack of standardized reporting criteria limits the extent to which their results can be directly compared with ours. This variability underscores the need for harmonized methodologies to enable more robust inter-study comparisons. Some studies report MFs per individual (e.g. Arias et al. 2019), while others report only per gram (e.g. Lopes et al. 2023). In studies that encompass both forms of reporting, values of MFs per gram are occasionally presented exclusively for individuals with MFs, omitting the total number of organisms studied (e.g. Santonicola et al. 2025). Within this framework, our results show that the abundance of MFs in *P. kreftti* was high in both seasons, even higher than those found in other notothenioids inhabiting the MPA N/BB (*P. ramsayi:* 2.80 ± 2.32 MFs ind.$^{-1}$ and *P. guntheri*: 2.18 ± 1.89 MFs ind.$^{-1}$; Ojeda et al. 2024). The species

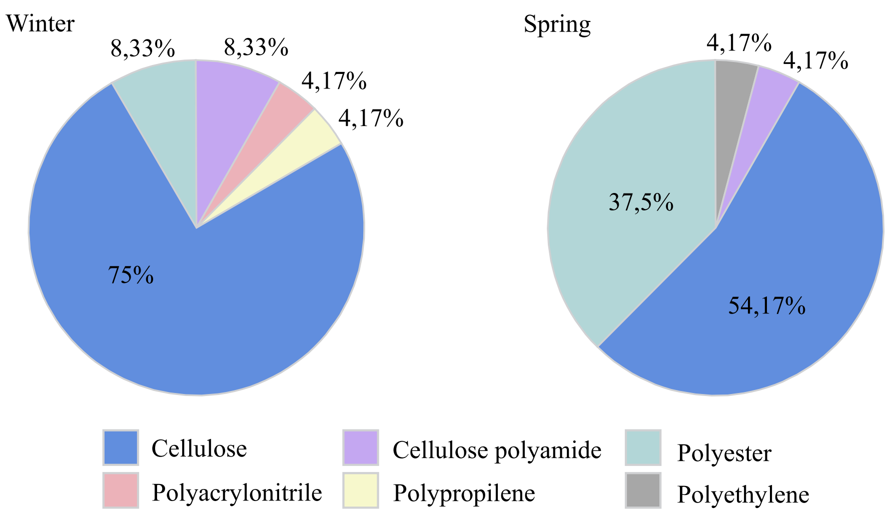

Figure 5. Chemical composition of microfibers found in digestive tracts of *Patagonotothen kreffti* during two seasons (winter and spring) of 2018 in the Marine Protected Area Namuncurá/Burdwood Bank.

of genus *Patagonotothen* play a key role in the trophic ecology of the southern Atlantic Ocean. These species consume a variety of benthic and supra-benthic invertebrates (Laptikhovsky 2004; Hüne and Vega 2016; Covatti Ale et al. 2022; Fischer et al. 2022) and serve as prey for a wide variety of fishes, mammals and seabirds (Brickle et al. 2006; Riccialdelli et al. 2013; Hüne and Vega 2016). Feeding habits of *P. kreffti* are unknown; however, it is assumed to exhibit generalist feeding behavior, similar to its sister species *P. ramsayi* (Álvarez Oyarzo 2020) and other members of the genus *Patagonotothen*. Fishes with generalist habits and prey variability are highly susceptible to haveing greater AP abundances, particularly MFs (Peters et al. 2017; Ferreira et al. 2019; Scacco et al. 2022; Ojeda et al. 2024), due to trophic transference. On the other hand, direct consumption of particles is feasible (Scacco et al. 2022). The MFs are the most abundant APs in the oceans (Cesa et al. 2017), particularly in the MPA N/BB, where a high abundance of MFs was documented, uniformly distributed throughout the water column (17.4 ± 12.6 MFs L^{-1}; Di Mauro et al. 2022).

The distribution of species belonging to the genus *Patagonotothen* extends across a considerable region of the southwestern Atlantic Ocean (Eastman and Eakin 2000). *Patagonotothen kreffti* records are limited to the MPA N/BB (Álvarez Oyarzo, 2020). Therefore, the APs acquired directly or indirectly could serve as indicators of the pollution level within the MPA N/BB.

The abundances of MFs in fish did not show significant differences between seasons. During winter, the BB water experiences intense convection that facilitates the transport of deep water to the upper layers (Matano et al. 2019). This process would favor the resuspension and availability of MFs in the water column. During the warm season, restratification has been shown to reduce mixing and increase the residence time of particles, as indicated by the formation of a density gradient (Matano et al. 2019), which could also lead to the retention of MFs. Previous studies conducted at the MPA N/BB on APs ingestion in *P. guntheri* and *P. ramsayi* (Ojeda et al. 2024) are in concordance with our findings in *P. kreffti*. Blue, black and red particles were predominant, with similar percentages. In addition, Di Mauro et al. (2022) found that the majority of the MFs in the water column were blue and black for the same region and seasons. Several authors in regions far from our study area have also reported the predominance of these colors in MFs (Zhang et al. 2021; Santonicola et al. 2024). There is evidence that blue MFs tend to be dominant in megafauna globally, both freshwater and marine (Gago et al. 2018; Macieira et al. 2021; Lopes et al. 2023; Alves et al. 2024) which can be attributed to its global use in denim jeans and its wear during washing (Athey et al. 2020). Besides, fish in marine environments tend to select APs with blue and black colors more frequently than other colors, suggesting that blue APs may be confused by fish due to similarly colored prey (Scacco et al. 2022), and also suggesting that other colors tend to be less stable once they enter the gastrointestinal tract of fish (Zazouli et al. 2022). The length of MFs varied in relation to their size, although 54% were shorter than 0.65 mm, which is consistent with what has been documented for other notothenioid species (Ojeda et al. 2024) and water column (Di Mauro et al. 2022) in the MPA N/BB. The relatively high proportion of aged (42% showed signs of wear and discoloration) and short MFs (52% < 0.65 mm) found in our study, supports the idea that much of the MFs in the MPA N/BB might be imported and driven by the Antarctic Circumpolar Current and even retained in the area for a long time (Matano et al. 2019; Di Mauro et al. 2022).

The chemical composition of the MFs in *P. kreffti* from both seasons is dominated by cellulose, as found in *P. ramsayi* and *P. guntheri* (Ojeda et al. 2024) and in sea stars *Henricia obesa* (Sladen, 1889) and *Odontaster penicillatus* (Philippi, 1870) from MPA N/BB (Cossi et al. 2021). Recent studies from other regions also found high proportions of cellulosic MFs. Natural and regenerated cellulosic MFs were found in proportions ranging from 55% to 72% in commercial species from the Tyrrhenian Sea (Santonicola et al. 2024), 78-82% in species from the Adriatic Sea (Santonicola et al. 2025), and 67.7-72.7% in fish from the southeastern Pacific and Antarctic coasts (Ergas et al. 2023). MFs originate from textile industry, industrial and domestic washing, and enter the oceans through waterways (Singh et al. 2020; Suaria et al. 2020) as well as atmospheric propagation (Xiao et al. 2023). Among synthetic polymers found, polyester stands out and is one of the most abundant types of APs in marine environments (Kanhai et al. 2016; Bessa et al. 2018) and is also found in high proportion in various fish (Bessa et al. 2018; Santonicola et al. 2024, 2025). The variety of chemical composition found in winter and of compounds with densities higher than water, such as polyamide and acrylic blends, is consistent with what was found in the previous study by Ojeda et al. (2024), where *P. ramsayi* from winter season presents a greater variety of polymers. The density of APs in the water column determines their bioavailability (Barletta et al. 2020). Stratification in the warm season could influence a lower variety of polymers in the water column, while in winter, strong convection would increase the availability of different density particles. This could be ingested indirectly by a variety of prey or, through the accidental ingestion of particles in water or sediments (Ferreira et al. 2019; Scacco et al. 2022; Ojeda et al. 2024).

Compounds found in notothenioids such as polyacrylonitrile (PAN), polyurethane and resins are considered polymers of higher toxicity (Yuan et al. 2022). Numerous studies in fish have detailed the effects of APs in fish and the risk to human health (e.g. Barboza et al. 2018, Barboza et al. 2020; Matias et al. 2024). Fish act as the main pathway for transporting APs to higher trophic levels, posing a potential threat to the entire ecosystem (Yuan et al. 2022). Findings of the present study suggest that species such as *P. kreffti* are highly exposed to contamination by MFs, reflecting the global dispersion of APs and the potential for transfer of toxic compounds through the food web, being a risk to biota and human health. *Patagonotothen kreffti* has the potential to serve as an indicator species for assessing the presence of MFs in the MPA N/BB, given its endemic status and its higher values of MF abundance than other notothenioid species present in the area.

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Author contributions

Mariel Ojeda: conceptualization; methodology; formal analysis; investigation; writing-original draft; visualization; writing-review and editing. Paula Bianconi: methodology; formal analysis; writing-review and editing. Guido N. Rimondino: methodology; formal analysis; investigation; writing-review and editing. Cintia P. Fraysse: writing- review and editing. Claudia C. Boy: conceptualization; investigation; resources; supervision; writing-review and editing. Analía F. Pérez: conceptualization; methodology; investigation; resources; writing-original draft; writing-review and editing; supervision; project administration, funding acquisition.

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